REMARKS

These remarks are in response to the Office Action dated February 28, 2002. Claims 1, 2, 5, 6, 9-13, 25, 26, 28-46 and 50 have been amended. Claims 4, 8 and 27 have been canceled without prejudice. Support for the amended claims can be found throughout the specification. No new matter has been added. Attached is a marked-up version of the changes being made by the current amendment. Claims 1, 2, 3, 5, 6, 7, 9-26, 28-46 and 50 are pending and at issue. Applicants respectfully request reconsideration of the present application.

I. REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Enablement

Claims 1-46 and 50 stand rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to enable one of skill in the art to make or use the invention. Applicants note that this rejection is moot with regard to canceled claims 4, 8 and 27. Applicants respectfully traverse this rejection as it may apply to the amended claims.

Specifically, the Office Action states that the specification "does not disclose a method of screening genes that modulate polyglutamine toxicity in any and [all] animals and any and all invertebrates using the method of the invention." Applicants note that transposable elements are extremely widespread throughout the animal world. For example, the transposable element designated "mariner" is present in organisms as diverse as hydra and humans. The present invention provides methods and animals for identifying genes that modulate

polyglutamine toxicity. The methods and animals are therefore useful for identifying genes or other compounds that modulate cellular and tissue degeneration and cell survival, for example, in neural, muscle, mesoderm, kidney and other tissues associated with frontotemporal dementia, prion diseases, polyglutamine disorders and protein aggregation disorders. Applicants believe that a person skilled in the art could easily make and use the claimed invention without undue experimentation in view of the scope of the pending claims. However, to advance prosecution, Applicants have amended claim 1 to recite "Drosophila." Support for the amendment may be found throughout the specification.

With regard to the Examiner's assertion that the claims are enabled only for *Drosophila melanogaster*, Applicants note that Engels et al., ("The origin of P elements in Drosophila melanogaster," 1992, BioEssays 14:681-686) have clearly shown that the P family of transposable genetic elements can be found in numerous Drosophila species, including *equinoxilis, paulistorum, pavloskiana, tropicalis, willistonis, fumipennis, nubulosa, sturtevanti, austrosaltans, lusaltans, prosaltans and saltans*. While claim 1 does not recite a "P transposable element," Applicants wish to note for the record that the presence of the P transposable element is not limited to the *melanogaster* species of the genus Drosophila.

The Office Action further indicates that the claims are only enabled for the use of a "P transposable element." Applicants note that the test of enablement is whether one skilled in the art could make or use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation. Applicants submit that as many as 30 classes of transposons have been found in

Drosophila. For example, in addition to P transposable elements, members of the "copia" class of transposable elements have been identified as facilitating many of the classical Drosophila mutations resulting from transposon insertion. Such transposable elements provide the means to develop vectors that modulate expression of a given gene in a particular tissue through fusion of the gene to a specific promoter. For example, a vector can include a promoter for strong expression in the developing egg and early embryo and a reporter gene for identifying those cells where the reporter molecule is expressed. Applicants have provided at least one representative example of a method that successfully used such a vector to modulate the expression of genes that decrease the effects of polyglutamine toxicity. In view of level of skill, state of the art and the information in the specification, Applicants submit that vectors comprising alternative transposable elements capable of modulating gene expression in Drosophila could be used without undue experimentation. Thus, the present invention should not be limited solely to the use of a P transposable element.

Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, first paragraph be withdrawn.

II. REJECTIONS UNDER 35 U.S.C.§112, SECOND PARAGRAPH

Claims 4-6, 8, 9, 21, 33 and 50 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants note that this rejection is moot with regard to

canceled claims 4 and 8. Applicants respectfully traverse this rejection as it may apply to the amended claims.

Claim 5 is allegedly indefinite for the recitation of "genus Drosophila melanogaster." Claim 5 has been amended to recite, in part, "wherein the Drosophila is Drosophila melanogaster."

Claim 6 is allegedly indefinite for the recitation of "wherein the marker sequence comprises a P element." Claim 6 has been amended to recite, in part, "wherein the transposable element comprises a P transposable element."

Claim 9 is allegedly indefinite for the recitation of "one or more of the near genes." Claim 9 has been amended to recite, in part, "the inducible upstream activating sequence increases or decreases expression of one or more operationally-associated gene(s)."

Claims 21 and 33 are allegedly indefinite for the recitation of "expression … is conferred by a constitutive, regulatable or tissue specific expression control element." Applicants submit that the passage clearly indicates that expression of a gene can be obtained via a 1) constitutive OR 2) regulatable OR 3) tissue specific "expression control element." Thus, Applicant believes that the claims are sufficiently definite because the claims do not attempt to encompass a single expression control element that is both constitutive and regulatable at the same time.

Claim 50 is allegedly indefinite for the recitation of "a length sufficient to produce polyglutamine toxicity." As noted on page 2, lines 7-9, "the relative length of the polyglutamine tract determines the aggregation propensity and cytotoxicity; the longer it is, the more likely it is to form inclusions and

cause cell death." Thus, the plurality of CAA and CAG sequences encoding a polyglutamine sequence need only encode a sufficient number of glutamines to produce polyglutamine toxicity. Applicants note that claim 50 is limited to those Drosophila exhibiting polyglutamine toxicity. Thus, the number of residues is variable only to the extent that the toxicity induced in the claimed Drosophila is detectable.

Claim 50 is allegedly indefinite for failing to recite a fertilization step. Applicants note that claim 50 has been amended to recite a "fertilized egg."

Claim 50 is allegedly indefinite for failing to recite an implantation step. Applicants note that claim 50 has been amended to recite a "Drosophila." Applicant submits that implantation of a Drosophila embryo is not necessary to complete the development process. Thus, it is unnecessary for claim 50 to recite such a limitation.

In view of the amendments to the claims and in light of the above discussion, Applicants respectfully request that the rejections under 35 U.S.C. §112, second paragraph be withdrawn.

II. REJECTIONS UNDER 35 U.S.C.§103

Claims 1, 4-16, 20-35, 41, 42 and 50 stand rejected under 35 U.S.C. §103 as allegedly obvious over Warrick and Tsubota. Applicants note that this rejection is moot with regard to canceled claims 4, 8 and 27. Applicants respectfully traverse this rejection as it may apply to the amended claims.

Specifically, the Office Action alleges that it was well known in the art of Drosophila genetics that P element-mediated hybrid dysgenesis is one of the very common modes of studying Drosophila genetics and control of gene expression, and that it

is very useful for identifying genes controlling various phenotypes in Drosophila. To establish prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. Further, if an independent claim is nonobvious under 35 U.S.C. §103, then any claim depending therefrom is nonobvious.

Warrick may teach the production of a Drosophila containing polyglutamine sequence and exhibiting polyglutamine toxicity, but Warrick fails to teach or suggest breeding such a Drosophila with a second Drosophila having a marker sequence inserted into its germline comprising 1) an inducible upstream activating sequence, 2) a minimal promoter sequence and 3) 5′ and 3′ transposable elements. Warrick further fails to teach or suggest producing progeny from the breeding of the first Drosophila with the second Drosophila. Finally, Warrick similarly fails to teach or suggest identifying genes operationally-associated with the marker sequence and conferring increased or decreased polyglutamine toxicity in the progeny having increased or decreased polyglutamine toxicity.

Tsubota does not remedy the deficiencies of Warrick. Tsubota is a general reference which confirms that gene expression can be studied using P element-mediated germ line transformation. However, the cited reference fails to teach the claimed invention for the reasons stated above.

Moreover, neither Tsubota nor Warrick suggest the desirability of combining the references. Even if presented with the cited references in combination, it is unclear how the skilled artisan would arrive at the claimed invention because neither reference provides a bridge that would lead to the claimed subject matter.

The conclusion that claimed subject matter is prima facie obvious must be supported by evidence, a showing by some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references to arrive at the claimed invention. At most, the combination of references suggests what may be obvious to try. "Obvious to try" has long been held not to constitute obviousness.

In summary, the combination Warrick and Tsubota does not render Applicants' claimed method and Drosophila obvious. Accordingly, Applicants respectfully request that the rejections under 35 U.S.C. §103 be withdrawn.

In summary, for the reasons set forth herein, Applicants maintain that claims 1, 2, 3, 5, 6, 7, 9-26, 28-46 and 50 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending. If the Examiner would like to discuss any of the issues raised in the Office Action, Applicants' representative can be reached at (858) 678-5070. Please charge any additional fees, or make any credits, to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 6/26/02

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Version with markings to show changes made

In the specification:

Please amend the paragraph beginning at page 1, line 20, and bridging to page 2, line 2, as follows:

--Expansion of polyCAG tracts is associated with human hereditary neurodegenerative disorders and neuronal toxicity (Kaytor *et al.*, *J. Biol. Chem.*, **274**:37507-37510 (1999)). Huntington's disease and several other hereditary neurodegenerative disorders are characterized by expansion of a polyglutamine sequence (LaSpada *et al.*, *Nature*, **352**:77-79 (1991); Koide *et al.*, *Nat. Genet.*, **6**:9-13 (1994); Kawaguchi *et al.*, *Nat. Genet.*, **8**:221-228 (1994); Orr *et al.*, *Nat. Genet.*, **4**:221-226 (1993); Sanpei *et al.*, *Nat. Genet.*, **14**:277-284 (1996); and Zhuchenko *et al.*, *Nat. Genet.*, **15**:62-69 (1997)). The expanded polyCAG tracts encode abnormally long polyglutamine sequences within specific proteins promoting their nuclear and/or cytoplasmic aggregation. The protein aggregation is believed to contribute to cellular toxicity including cell death or apoptosis (Trottier *et al.*, *Nature,* **378**:403-406 (1995); Davies *et al.*, *Cell*, **90**:537-548 (1997); and DiFiglia *et al.*, *Science*, **277**:1990-1993 (1997)).—

Please amend the paragraph at page 6, lines 21-30, as follows:

Thus, a transgenic animal of the invention including a transgene containing a plurality of CAGs and at least one CAA sequence encoding a polyglutamine repeat sequence can express a polyglutamine repeat sequence of any length. In one embodiment, the polyglutamine sequence is between about 5 and 20 amino acids in length. In another embodiment, the polyglutamine sequence is between about 20 and 50 amino acids in length. In yet another embodiment, the polyglutamine sequence is between about 50 and 100 amino acids in length. In additional embodiments, the polyglutamine sequence is between about 100 and 200 amino acids in length, between about 100 and 500 amino acids in length and between about 50 and 200 amino acids in length. In various aspects, a polyglutamine sequence further includes a tag (e.g., epitope, hemagluttinin, etc.).

Please amend the paragraph at page 35, line 30, and bridging to page 36, line 2, as follows:

The TPR2 gene corresponds to a cDNA of 2239 nucleotides. The MLF gene corresponds to a cDNA of 1753 nucleotides. Specifically disclosed herein are nucleic acid sequences for *Drosophila* TPR2 and MLF (SEQ ID NO:2 and SEQ ID NO:4, respectively; Figures 9 and 10).

Please amend the paragraph at page 73, lines 1-20, as follows:

Plasmid rescue (Pirrotta (1986); Pirrotta, Cloning Drosophila Genes: A Practical Approach, pp 83-110, IRL Press, Oxford, Washington, D.C., ed. D.B. Roberts (1986)) was done with the following modification: from an established line, genomic DNA was isolated by QIAamp Tissue kit (Qiagen) and digested with 6 restriction enzymes: BfrI, BglI, EcoRI, HincII, SacI, and SacII in 100µl reaction volume overnight. Digested fragments were purified by QIAprep Spin Miniprep kit (Qiagen), circularized by ligation in 50 µl reaction and transformed by electroporation of 1.5 µl of ligation reaction into the DH10B (Gibco/BRL) strain of *E. coli*. Colonies carrying the P-element were selected by plating transformed bacteria on media with Kanamycin. DNA was isolated from positive colonies and the approximate size of the insert (flanking genomic DNA) determined by AvaI restriction enzyme digestion. Inserts of sufficient size were sequenced by automated sequencing and the results were compared with known DNA or protein sequences in the database by Berkeley Drosophila Genome Project (BDGP) BLAST server (BLASTN) and The Baylor College of Medicine Search Launcher (BLASTP+BEAUTY). Protein alignments were performed by MacVector PPC 6.0.1 application software. Program parameters for *Drosophila* dTPR2 and human TPR2 were Clustal W(1.4), Pairwise alignment mode: slow: Open Gap penalty 10.0: Extend gap penalty 0.1; similarity matrix blosum. For *Drosophila* dMLF and human MLF the program parameters were Clustal W(1.4), Pairwise alignment mode: slow: Open Gap penalty 1.0: Extend gap penalty 0.1; similarity matrix blosum. EST search parameters were BLASTN 2.0a19MP.

In the claims:

Claims 4, 8 and 27 have been cancelled.

Claim 1, 2, 5, 6, 9-13, 25, 26, 28-46 and 50 have been amended as follows:

1. (Amended) A method of screening for genes that modulate polyglutamine toxicity comprising:

(a) providing a first [animal] Drosophila expressing a polyglutamine sequence, wherein the sequence produces polyglutamine toxicity in the [animal] Drosophila;

(b) breeding the first [animal] Drosophila to a second [animal] Drosophila, wherein the second [animal] Drosophila has a marker sequence inserted into its germline, wherein the marker sequence comprises 1) an inducible upstream activating sequence, 2) a minimal promoter sequence and 3) 5′ and 3′ transposable elements;

(c) [thereby] producing progeny from the breeding of the first Drosophila with the second Drosophila;

([c]d) screening the progeny for increased or decreased polyglutamine toxicity relative to the first [animal] Drosophila thereby identifying a progeny having increased or decreased polyglutamine toxicity; and

([d]e) identifying one or more genes [adjacent to] operationally-associated with the marker sequence, or having an insertion of the marker sequence, that confers increased or decreased polyglutamine toxicity in the progeny having increased or decreased polyglutamine toxicity.

2. (Amended) The method of claim 1, further comprising [step (e),] identifying a mammalian homologue of the gene of claim 1.

3. (Reiterated) The method of claim 2, wherein the mammalian homologue comprises a human homologue.

4. (Canceled) The method of claim 1, wherein the first and second animals are invertebrates.

5. (Amended) The method of claim [4] 1, wherein the [invertebrates are of the genus] Drosophila is Drosophila melanogaster.

6. (Amended) The method of claim 1, wherein the [marker sequence] transposable element comprises a P transposable element.

7. (Reiterated) The method of claim 1, wherein the marker sequence comprises a polynucleotide sequence that disrupts or alters expression of one or more genes near the sequence.

8. (Canceled) The method of claim 1, wherein the marker sequence further comprises an expression control element conferring expression of the one or more genes near the marker.

9. (Amended) The method of claim [8] 1, wherein the [expression control element] inducible upstream activating sequence increases or decreases expression of one or more [of the near] operationally-associated gene(s).

10. (Amended) The method of claim 1, wherein the second [animal] Drosophila is selected from a group of two or more

animals having markers inserted into different locations of its genomic DNA.

11. (Amended) The method of claim 10, wherein the second [animal] Drosophila is selected from a group of 10 to 100, 100 to 500, or 500 or more of the animals.

12. (Amended) The method of claim 1, wherein the second [animal] Drosophila is selected from a library of animals having markers inserted at random locations of their genomic DNA.

13. (Amended) The method of claim 12, wherein the library of [animals] Drosophila is generated by random P element insertion.

14. (Reiterated) The method of claim 1, wherein the polyglutamine sequence comprises a sequence having between about 35 to 50, or between about 50 to 100 glutamine residues.

15. (Reiterated) The method of claim 1, wherein the polyglutamine sequence comprises a sequence having between about 100 to 150 glutamine residues.

16. (Reiterated) The method of claim 1, wherein the polyglutamine sequence comprises a sequence having about 150 or more glutamine residues.

17. (Reiterated) The method of claim 1, wherein the polyglutamine sequence further comprises a tag.

18. (Reiterated) The method of claim 17, wherein the tag comprises an epitope tag.

19. (Reiterated) The method of claim 18, wherein the epitope tag comprises a hemagglutinin sequence.

20. (Reiterated) The method of claim 1, wherein the polyglutamine sequence is encoded by a polynucleotide containing a plurality of CAGs, CAAs or a combination thereof.

21. (Reiterated) The method of claim 20, wherein expression of the plurality of CAGs, CAAs or combination thereof is conferred by a constitutive, regulatable or tissue specific expression control element.

22. (Reiterated) The method of claim 21, wherein the regulatable element comprises an inducible or repressible element.

23. (Reiterated) The method of claim 21, wherein the regulatable element comprises a GAL4 responsive sequence.

24. (Reiterated) The method of claim 21, wherein the tissue specific element confers neural, retinal, muscle or mesoderm cell expression.

25. (Amended) A progeny [animal] Drosophila produced by the method of claim 1.

26. (Amended) A transgenic [animal] Drosophila comprising a transgene containing a plurality of CAG's and at least one CAA sequence encoding a polyglutamine repeat sequence.

27. (Canceled) The animal of claim 26, wherein the animal is an invertebrate.

28. (Amended) The [animal] Drosophila of claim [27] 26, wherein the [invertebrate animal is] Drosophila is Drosophila melanogaster.

29. (Amended) The [animal] Drosophila of claim 26, wherein the number of CAG's to CAA's is in ratio of between about 1:1 and 2:1.

30. (Amended) The [animal] Drosophila of claim 26, wherein the number of CAG's to CAA's is in ratio of between about 2:1 and 5:1.

31. (Amended) The [animal] Drosophila of claim 26, wherein the number of CAG's to CAA's is in ratio of between about 5:1 and 10:1.

32. (Amended) The [animal] Drosophila of claim 26, wherein the number of CAG's to CAA's is in ratio of between about 10:1 and 50:1.

33. (Amended) The [animal] Drosophila of claim 26, wherein expression of the polyglutamine sequence is conferred by a

constitutive, regulatable or tissue specific expression control element.

34. (Amended) The [animal] Drosophila of claim 33, wherein the tissue specific expression control element confers neural, retinal, muscle or mesoderm cell expression.

35. (Amended) The [animal] Drosophila of claim 33, wherein the tissue specific expression control element comprises an Appl or rhodopsin 1 promoter or GLASS transcription factor element.

36. (Amended) The [animal] Drosophila of claim 26, wherein the polyglutamine sequence is between about 30 and 50 amino acids in length.

37. (Amended) The [animal] Drosophila of claim 26, wherein the polyglutamine sequence is between about 50 and 100 amino acids in length.

38. (Amended) The [animal] Drosophila of claim 26, wherein the polyglutamine sequence is between about 100 and 200 amino acids in length.

39. (Amended) The [animal] Drosophila of claim 26, wherein the polyglutamine sequence is between about 50 and 200 amino acids in length.

40. (Amended) The [animal] Drosophila of claim 26, wherein the polyglutamine sequence further comprises a tag.

41. (Amended) The [animal] Drosophila of claim 26, wherein polyglutamine toxicity is produced in one or more tissue or organs of the animal.

42. (Amended) The [animal] Drosophila of claim 26, wherein the animal further comprises a marker sequence inserted into its genomic DNA, wherein the marker is located adjacent to a gene or inserted into a gene whose expression or activity increases or decreases polyglutamine toxicity in the animal, and wherein the marker sequence comprises an inducible upstream activating sequence, a minimal promoter sequence and 5′ and 3′ transposon elements containing terminal inverted repeats.

43. (Amended) The [animal] Drosophila of claim 42, wherein the marker sequence is near or inserted into a gene containing a J domain.

44. (Amended) The [animal] Drosophila of claim 43, wherein the gene is HDJ1.

45. (Amended) The [animal] Drosophila of claim 43, wherein the gene is TPR2.

46. (Amended) The [animal] Drosophila of claim 43, wherein the marker sequence is near an MLF gene.

50. (Amended) A method of producing a transgenic [animal] Drosophila characterized by polyglutamine toxicity comprising:

(a) transforming a[n animal] Drosophila embryo or fertilized egg with a transgene comprising a plurality of CAA

and CAG sequences encoding a polyglutamine sequence having a length sufficient to produce polyglutamine toxicity in the [animal] Drosophila produced from the embryo or fertilized egg; and

(b) selecting a[n animal] Drosophila that exhibits polyglutamine toxicity in one or more cells or tissues.